DISSERTATION ON

SPLIT SKIN GRAFTING

versus

MINIATURE PUNCH GRAFTING

IN VITILIGO

This dissertation is submitted to

THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY

In partial fulfilment of the requirement of the award for the degree of

M.D BRANCH XX

DERMATOLOGY, VENEREOLOGY AND LEPROSY

STANLEY MEDICAL COLLEGE

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DECLARATION

I solemnly declare that the dissertation titled**, Split Skin Grafting versus Miniature punch Grafting in Vitiligo** was done by me at **Stanley Medical College and Hospital during 2009-2012** under the guidance and supervision of my **Chief Prof Dr. K. Manoharan, M.D., D.D**

The dissertation is submitted to **THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY** towards the partial fulfilment of requirement for the award **of M.D. Degree (Branch XX) in DERMATOLOGY, VENEREOLOGY & LEPROSY.**

Place:

Date

DR. T.Vanathi.

CERTIFICATE

This is to certify that the dissertation titled **'SPLIT SKIN GRAFTING VERSUS MINIATURE PUNCH GRAFTING IN VITILIGO'** is submitted by **Dr. T.Vanathi** to The Tamilnadu Dr. M. G. R Medical University, Chennai in partial fulfilment of the requirement of the award for the degree of **M.D BRANCH XX (DERMATOLOGY, VENEREOLOGY AND LEPROSY)** and is a bonafide work done by her under direct supervision and guidance.

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CONTENTS

INTRODUCTION

INTRODUCTION- VITILIGO SURGERIES

The main intention of the any vitiligo surgery is to repigment the vitiligenous areas with a natural colour of the individual by various means:

- Simulating the natural colour by tattooing[1], a method in which artificial pigments are introduced by microneedling technique.
- Using a thin Theirsch's[2] graft to cover the denuded achromic areas.
- Various grafts, e.g. ultra-thin grafts[3], suction blister[4] and miniature punch grafts[5], non-cultured epidermal cell suspension or transplantation, and epidermal[6] and melanocyte cultures[7] can be used to repopulate the decreased melanocytes.
- The lesional melanocytes can be stimulated from the periphery and the black hair follicles to proliferate, migrate and re-pigment the lesion by Therapeutic wounding[8] e.g. therapeutic dermabrasion, laser ablation, cryosurgery (liquid nitrogen spraying), needling, and local application of phenol or trichloroacetic acid.

AIM AND OBJECTIVES OF THE STUDY

1. To compare the effect of split skin grafting versus miniature punch grafting in vitiligo patients.
2. To look for the adverse effects during these procedures.

REVIEW OF LITERATURE

History[12]:

The origin of the word vitiligo has many proposed meanings. The Rigveda (6000 BC or earlier) named it as Kilas, meaning white spotted deer[9]. In Buddhist sacred books also it was named as Kilas. Some believed it to be originated from `vitelius' meaning vale, i.e. pale pink flesh of calf because of its clinical resemblance to the white patches of the spotted calf. Few others think it has its origin in Latin word `vitium' meaning blemish.

Epidemiology:

Vitiligo affects almost all races in the world. The worldwide incidence ranges from 0.1 to 1.3%[10,11]. In India it varies from 3-4% to as high as 8.8%.

Definition[12]:

Vitiligo is an acquired progressive melanocytopenia of unknown etiology, clinically manifested by circumscribed achromic macules often

associated with leukotrichia and histologically by degeneration and disappearance of melanocytes in the involved skin and not infrequently in the pigment epithelium of the eyes, leptomeninges and inner ear.

Etiopathogenesis:

THE CLASSIC HYPOTHESES:

Autoimmune theory:

This is the most popular theory citing that melanocytes are killed by autoimmune effector mechanisms. The association of other autoimmune disorders like pernicious anemia, autoimmune thyroiditis, Addison's disease supports this theory[13]. The first clue for participation of cellular immunity in vitiligo pathogenesis was the discovery of T-cell infiltrate in the margin of the inflammatory vitiligo. Following successful immunotherapy of melanoma, vitiligo like depigmentation has been noted. Humoral immunity too has a role and various circulating autoantibodies against Tyrosinase, TRP-1, TRP-2, etc. are found with their titres well correlating with disease activity.

The Neural Hypothesis:

This theory suggests that destruction of melanocytes due to alteration in the ratio of the neurotransmitters or due to liberation of unusual neurotransmitter. The occurrence of vitiligo in segmental pattern and anecdotal reports of its origin after viral encephalitis, following peripheral nerve injury, its association with multiple sclerosis and Horner`s syndrome further supports this. Communication between nervous system and epidermal melanocytes has been proved[14].

The autocytotoxic theory:

Lerner[15],in the year 1971 postulated that melanocytes have a genetically based protective mechanism that eliminates toxic products like DOPA, DOPAchrome and 5,6-dihydroxyindole which are produced during the synthesis of Melanin. Some people who are deficient in these mechanisms have accumulation of these melanotoxic products, which result in depigmentation.

THE NEW HYPOTHESES:

A disorder of melanocyte survival:

The involvement of melanocyte apoptosis and of SCF[16]/c-kit/MITF/Bcl-2 pathway in the pathogenesis of vitiligo indicates their key role in the maintenance of melanocyte survival.

a) SCF from keratinocytes strongly protects melanocytes from TNF-related apoptosis inducing ligand(TRAIL)
b) Bcl-2, a MITF-dependent kit transcriptional target in melanocytes, is essential for maintaining the lifetime of melanocytes. When Bcl-2 expression is decreased, the susceptibility of the melanocytes to apoptosis increases.
c) Down regulated expression of c-kit and MITF-M proteins at the edges of lesional vitiligo epidermis have been confirmed by Western blotting,

The melanocyte growth factor deficiency theory:

Partial correction of the defective growth and passage capacities of vitiligo melanocytes by the adjunction of fetal lung fibroblasts-derived growth

factors suggests that the decreased concentration of melanocyte growth factor(s) could play a role in the pathogenesis of vitiligo[17].

Melanocyte defective adhesion:

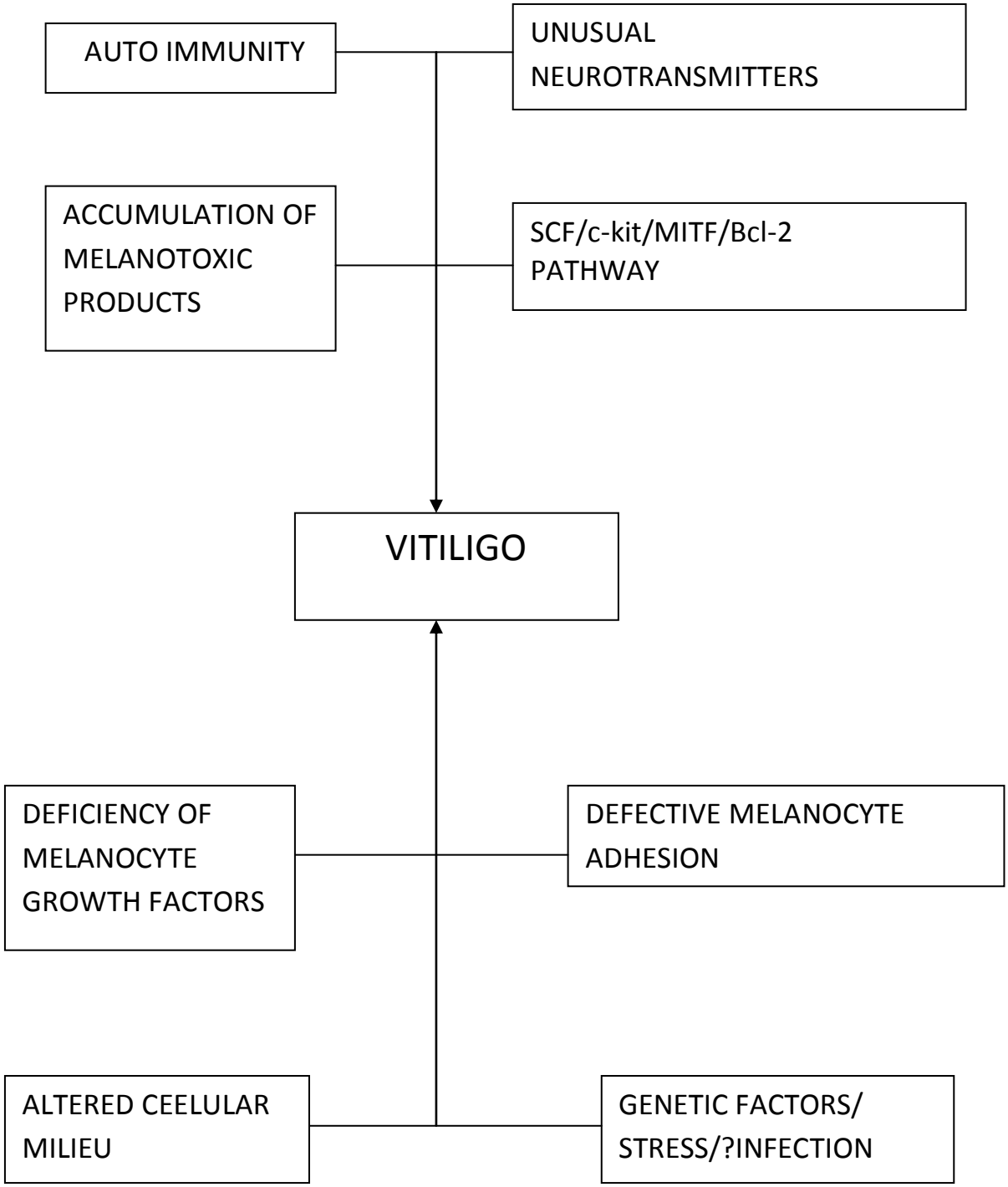
Interaction between melanocytes and basement membrane zone are mediated by α6β1 integrins. Cadherins and β-catenin helps the interaction between the keratinocytes and melanocytes. Repeated friction in non-lesional skin of vitiligo patients induces detachment and transepidermal elimination[18] of melanocytes indicating the probable cause of koebnerization.

All hypotheses are not mutually exclusive.

Consequence theory[19]:

It suggests that genetic factors, stress, accumulation of toxic compounds, autoimmunity, infection, altered cellular environment and impaired melanocyte migration and proliferation can all contribute to the phenomenon of vitiligo.

COMPOSITE THEORY OF PATHOGENESIS[12]



Understanding the mechanism of repigmentation in vitiligo:

The nature of repigmentation can be classified into three types:

a) Perifollicular when predominant repigmentation is follicular
b) Marginal, when the predominant repigmentation is from the border of the patches
c) Diffuse pigmentation when there occurs generalized darkening across the patches of the vitiligo.

MECHANISMS OF MEDICALLY INDUCED REPIGMENTATION:

PERIFOLLICULAR REPIGMENTATION[20]:

Three stages:

1) Proliferation of hypertrophic melanocytes in the lower portion of hair follicle
2) Migration of hypertrophic melanocytes along the hair follicle toward the infundibulum
3) Migration of melanocytes to the adjacent epidermis

The melanocyte mitogens- basic fibroblast growth factor, stem cell factor and endothelin-1 have been shown to stimulate melanocyte migration which may be either chemotactic or chemokinetic. Matrix metalloproteinases are found to be involved in extracellular matrix

remodeling and in cell migration during physiological and pathological processes.

Diffuse repigmentation:

Jarrett and Szabo[21] suggested that reactivation of dihydroxyphenylalanine (DOPA) negative melanocytes which persist in the center of lesions might be responsible for diffuse type of repigmentation.

Marginal repigmentation[19]:

A topically applied drug has chemo-attractant action for melanocytes, which attracts large reservoir of normal melanocytes surrounding the depigmented patch of vitiligo. Those melanocytes gradually migrate and actively produce melanin.

Mechanisms of surgically induced repigmentation:

The aim of surgical induction of repigmentation is

To replenish melanocytes in the depigmented lesions of vitiligo which have no reservoir or which fail to activate melanocyte in the outer root sheath with known treatment modalities[19].

The various mechanisms by which this happens are

1) Altered milieu of epidermal cytokines caused by a simple dermabrasion can stimulate inactive melanocytes of outer root sheath. These melanocytes which are seen in the epidermis 8-10 days after dermabrasion are 2-3 times larger than normal melanocytes with a hypertrophic cellular body, elongated dendrites, and intense DOPA oxidase activity.
2) In minigrafting, the tiny miniature grafts acts as islands of melanocyte reservoir[19], which stimulates hair follicle reservoir. The Perigraft pigmentation originating from these minigrafts spread generally 5-10 mm beyond the graft margin.
3) The ability of the melanocytes to migrate plays a role in surgical grafting either through suction blister grafting or epidermal grafting. The graft take is not necessary for successful

repigmentation[22]. The contact of graft with denuded recipient vitiligenous area for 7-8 days causes melanocyte migration from the graft to graft bed and causes success.

4) In transplantation of melanocyte-keratinocyte cell suspension or in cultured “pure” melanocytes, diffuse repigmentation occurs because of even repopulation of melanocytes to the recipient area[19].

Classification of vitiligo:

Broadly vitiligo can be classified into vitiligo vulgaris and vitiligo pseudosegmentalis.

According to the distribution of lesions it can be divided into localized and generalized type.

Table 1 Classification of vitiligo according to the distribution of lesions[19]

1. Localized
   (a) Focal
   (b) Segmental
   (c) Mucosal
2. Generalized
   (a) Acrofacial
   (b) Vulgaris
   (c) Universal
   (d) Mixed

Segmental type is usually localized to one dermatome, shows relatively stable

disease activity after its initial rapid-spreading phase, and is associated with a significantly lower rate of autoimmune diseases than non-segmental type.

Table 2 Classification of vitiligo with emphasis on segmental vitiligo[19].

1. Segmental (unilateral)
2. Non-segmental (bilateral)

(i) Localized

(a) Focal

(b) Mucosal

(ii) Generalized

(a) Acrofacial

(b) Vulgaris

(c) Universal

3. Mixed: segmental and non-segmental

Clinical features of non-segmental (bilateral) vitiligo:

This type includes all types of vitiligo other than segmental.

Focal vitiligo:

This type exhibits one or more macules in one area, but not clearly in a segmental or zosteriform distribution. Focal vitiligo is a starting point leading to other types of vitiligo. Without treatment it frequently spreads to the whole body. However early treatment will not guarantee prevention of its spread. Systemic steroids effectively prevent the spread of this type.

Mucosal vitiligo:

Vitiligo of lips, gingival, genitals, areolae and nipples occur in this type. Significant progression of vitiligo in patients with mucosal involvement indicates poor prognosis.

Acrofacial vitiligo:

This encompasses vitiligo lesions in the distal part of the extremities (hands, feet) and circumferential lesions around facial orifices.

Vitiligo vulgaris:

This entity is composed of several scattered macules.

Universal vitiligo:

In this type loss of pigment is seen >80% body surface.

Clinical features of Segmental vitiligo:

This has an earlier onset and spreads rapidly within the affected area. But it is limited to one segment example: face, part of trunk and extremity or one extremity. The lesions stop abruptly at the midline of the affected segment. After the cessation of the progression the vitiligo patch remain without any change for the life of the patient. Very rarely it can progress again after being quiescent for many years. Studies on its distribution show it most commonly involves trigeminal nerve, followed by the thoracic, cervical, lumbar and sacral nerves. However the actual depigmentation will not correlate very well to a true dermatome. They may represent an unknown pathway of group of identical clonal cells.

Involvement of other pigment epithelium:

Destruction of the pigment epithelium in the retina and the outer choroids may give a tigroid appearance. Scarring too can occur and impairs visual acuity. The pigment epithelium of inner ear and leptomeninges can also get involved.

Associated skin disorders[12]:

Alopecia areata

Atopic eczema

Psoriasis

Scleroderma

Lichen planus

Lichen simplex

Discoid lupus erythematosus

Halo nevus

Ichthyosis vulgaris

Associated systemic disorders[12]:

Pernicious anemia

Addison`s disease

Grave`s disease

Hyperthyroidism

Hypothyroidism

Thyroiditis

Hyperparathyroidism

Diabetes mellitus

Internal malignancy

Factors affecting vitiligo progression:

Physical trauma

Sunburn

Psychological stress

Pregnancy

Contraceptives

Medical treatment:

Phototherapy:

This include photochemotherapy (oral and topical) such as psoralen plus UVA (PUVA), Psoralen plus sunlight (PUVASOL), broadband and narrowband UVB (BB- and NB-UVB), 308-nm excimer light and combination phototherapy.

UVA phototherapy:

Topical PUVA photochemotherapy:

This therapy is usually restricted to vitiligo patients with an involvement of less than 20% of the body surface[19]. One percent 8 methoxypsoralen (8-MOP) is diluted from 1:10 to 1:100. This should be applied only to the areas of depigmentation with a cotton tip applicator taking care that it should not drip to the normal areas. The patient is exposed to UVA approximately 20-30 minutes after application.

The initial dose should not exceed 0.25J/cm^2. This treatment is given once or twice a week. It should never be given on two consecutive days. Dose increments in the range of 0.12-0.25J/cm^2/week can be done,

until mild erythema is achieved at the treated sites. Following treatment, the area is washed thoroughly. A broad spectrum sunscreen is applied and excessive sun exposure should be avoided for at least 24 hours[23].

In PUVASOL therapy, where sunlight is used as a source of UVA there are more chance of blistering. In one study done by Grimes PE, only 3% had blistering reactions out of 125 patients who had home based PUVASOL therapy where very dilute 8-MOP (0.001%) was used.

Oral PUVA photochemotherapy:

PUVA involves the use of psoralens followed by exposure to long-wavelength UVA irradiation. Oral Trimethoxypsoralen (TMP) is preferred to 8-Methoxypsoralen (more phototoxic) and 5-Methoxypsoralen (less effective). The dose of it is 0.6mg/kg body weight ingested along with food. After 2 hours, the area is exposed to approximately 3 J/cm^2 of UVA for 5 minutes. The exposure time can be gradually raised until erythema beginning about 12-18 hours after exposure, and maximal at 48 hour is achieved after each exposure[12], with a twice a week treatment. If repigmentation is not seen even after 20-39 treatments of 45 minutes exposure to each site, the dose of TMP is increased to 0.9mg/kg body

weight. If the desired results are still not achieved after another 20-30 treatments, the drug is changed to 0.3mg/kg body weight of 8-MOP.

The irradiance of light is measured in milliwatts/cm^2 (mw/cm^2) with the help of an appropriately calibrated radiometer. The dose of UVA irradiance is expressed in joules/cm^2 (J/cm^2). The following formula is employed to calculate exposure time in minutes for the desired dose in joules.

$$\text{Exposure time (min)} = \frac{\text{Prescribed UVA dose } (J/cm^2)}{0.06 \text{ X Irradiance } (mw/cm^2)}$$

The following points should be kept in mind while on PUVA therapy:

- Treatment can be given twice or thrice a week, but never on two consecutive days.
- Therapy usually needs to be continued for 9-19 months.
- For satisfactory improvement it has been estimated at least 150-300 exposures may be needed.
- It should be avoided in patients less than 12 years of age, during pregnancy and lactation, and in patients with a past history of photosensitivity or X-ray therapy.

- ❖ Concurrent treatment with potential photosensitizing drugs should be avoided.
- ❖ Ophthalmological check up should be done initially and periodically.
- ❖ Hematological status, renal and hepatic functions are to be assessed initially and periodically during the period of therapy.

UVB therapy:

Broadband UVB lamps emit light in a broad range which can be helpful and also responsible for increased chance of phototoxicity and skin cancers.

Narrow band UVB has overcome all these side effects and is very effective[24]. The emission spectrum of NB-UVB is 311nm. The starting dose varies from 100 to 250 mJ/cm^2. Increments of about 10 to 20% can be done with each subsequent exposure.

The advantages of NB-UVB therapy are

- ❖ No oral medication needed so no side effects of Psoralen.
- ❖ Can be used in pregnancy and children.
- ❖ No post exposure eye protection is necessary.
- ❖ Exposure time is shorter than with PUVA.

Excimer laser:

The 308-nm xenon chloride excimer laser is found to be useful in chronic stable vitiligo, with studies claiming good results in short period of time. Its given twice or thrice weekly for 10-15 sittings. Initial pigmentation is seen within 4-8 weeks. But treatment for >12 weeks only gives satisfactory repigmentation.

Other modes of photochemotherapy includes

Khellin with UVA exposure

Phenylalanine with UVA exposure

Calcipotriol with PUVA therapy.

Corticosteroids:

Topical corticosteroids are the first line of treatment in patients with a few localized lesions. With mid potent and super potent topical corticosteroids like mometasone, betamethasone, clobetasol marked to complete repigmentation has occurred[25].

It is applied once or twice daily for 6-8 weeks followed by treatment free interval of several weeks. After 3 months if there is no

repigmentation, this treatment has to be stopped. The exact mechanism of steroid induced repigmentation is not known. The proposed hypotheses are may be due to suppression of immunity driven melanocyte destruction and stimulation of melanocyte proliferation and migration[19]. Side effects include skin atrophy, acne, ecchymosis, telengiectasia, rosacea and striae. To avoid these side effects steroids should be applied only for limited area and uninterrupted prolonged use should be avoided.

Systemic steroids- high dose pulse, oral mini pulse and daily oral low dose have been claimed to give good results in generalized vitiligo vulgaris. The long list of side effects of corticosteroids should always be borne in mind.

Calcineurin inhibitors:

Tacrolimus 0.1% and Pimecrolimus 1% ointments have been found to be effective in both localized and generalized vitiligo[26,27]. In a 2 month double-blind randomized trial, both tacrolimus and clobetasol have given equal results only[28]. But topical calcineurin inhibitors give good results in sun exposed areas[19].

Immunomodulators:

Levamisole, anapsos, Isoprinosine and suplatast were the immunomodulators with some preliminary studies showing some efficacy. Unfortunately, the initial observations in the efficacy of these compounds in the treatment of vitiligo have not been followed by clinical studies. As a consequence, these agents have not been widely used by vitiligo patients.

Others:

Antioxidants like tocopherols, ascorbic acid, ubiquinone and selenium methionine are widely prescribed by many dermatologists to arrest the spread of vitiligo and to promote its repigmentation. But till date the use of these agents are not validated by any controlled clinical trial.

Anecdotal report of claiming good repigmentation with topical prostaglandin E2 (PGE2)[29] have to be confirmed with further studies.

Surgical therapy:

In the middle of 20th century, Spencer and Tolmach[30] harvested punch grafts from normal skin and grafted it in to the vitiligenous skin in the year 1952. Following that there were many similar trials were done, but with inconsistent results. They were unable to predict who will repigment and who will not.

Various types of surgeries were tried and extensively studied. Basically five groups of procedures have been described since initial trials were done. They are

1) Thin dermo-epidermal grafts
2) Epidermal grafting
3) Minigrafting
4) Epidermal suspensions
5) In vitro cultures of epidermis with melanocytes and melanocyte suspensions

Thin split-thickness skin grafts:

This surgical technique was first introduced by Ollier in 1872 and Thiersch in 1874[2]. In this method, dermo-epidermal grafts harvested with

a knife or dermatome in vitiligo patients. Split thickness grafts are taken and grafted over dermabraded achromic vitiligenous areas.

Epidermal grafting:

In the year 1971, for the first time Falabella[4] prepared epidermal grafts by suction blistering and he succeeded in treating segmental vitiligo and depigmentation in post-burn injury. At present, epidermal grafting is one of the most popular techniques used with great success for vitiligo repigmentation.

Minigrafting (Miniature Punch grafting):

This technique was also described by Falabella[31] in 1983. This method was designed to simulate the pigmentation response occuring during perifollicular repigmentation in vitiligo after medical treatment, a method that is particularly successful for patients with stable vitiligo. Small minigrafts of 1-1.2mm were harvested with a punch from hidden donor sites, such as the gluteal region and implanted at the recipient sites, where similar perforations were made to prepare the recipient sites.

Epidermal suspensions:

This effective method was initially described by Gauthier and Surleve-Bazeille[32] in the year 1992. The epidermal suspension was obtained by exposing thin dermo-epidermal sheet to a 0.25% trypsin solution. After enzymatic digestion, the epidermal cells became separated individually forming a cell suspension that was seeded on the denuded achromic area.

In vitro cultures of epidermis with melanocytes and melanocyte suspensions:

In 1989, Falabella[6] et al cultured for the first time epidermis with melanocytes for the purpose of repigmentation. Epidermal layers successfully grown on Eagle`s minimal essential medium, supplemented with pituitary extract and hormones, were applied to patients with satisfactory results.

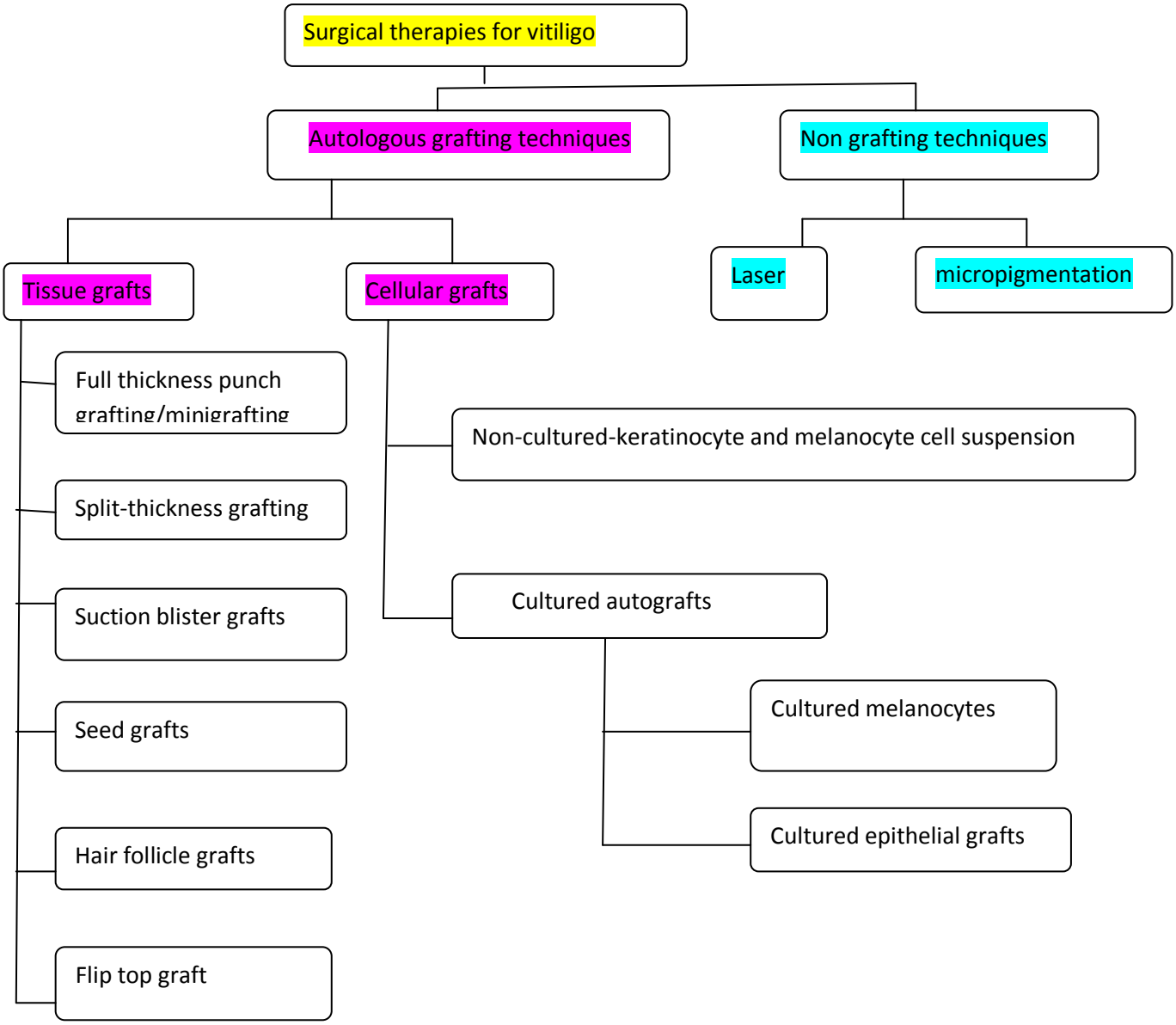


Figure 1- Classification of various surgical techniques employed in patients with vitiligo and other leukodermas[19].

MINIGRAFTS- Full thickness punch grafts:

Procedure:

A punch biopsy, encompassing both the epidermis and the dermis, is used as a graft.

Preparation of recipient site:

Small perforations are performed after local anaesthesia with a biopsy punch of 1.2-2.5mm. The perforations should be of 0.5-1mm deep and be separated from each other by 3-5mm. the achromic plugs are pulled out and the recipient area is covered with normal saline compresses. When the treated is large and many perforations are anticipated, a motor driven mini-punch device is helpful to prepare the recipient site. Pulsed Er:YAG (erbium: yttrium-aluminium-garnet) laser was proposed as an alternative to punch biopsy to prepare the recipient site.

Harvesting of grafts and grafting:

Perforations are performed with a punch of similar size as for recipient site, at a distance of about 1mm from each other. Some recommend taking a punch biopsy 0.25-0.5mm larger in diameter than the recipient site because of the retraction of the graft site. Grafts are freed from the donor site and excess adipose tissue is trimmed to achieve a thickness equal to the depth of recipient pits. Grafts are inserted into the small perforations, held in place with steri-strips and covered by a non-adherent dressing for approximately 1 week. Sunlight exposure or PUVA helps in stimulation of pigment spread.

Repigmentation around punch grafts usually starts 2-3 weeks after graft placement. Coalescence of pigment from all punch grafts occurs within 4-6 months. Good repigmentation has been achieved in 68-82% of cases.

Advantages:

Easiest

Least expensive

No special equipment

Suitable for difficult locations- lips, palms, soles and fingers

Disadvantages:

Time consuming for large areas

Regrafting between the transplanted grafts may be necessary

Not suitable for body folds

Split-thickness grafts:

Procedure:

A sheet of skin of 0.2-0.3 mm thickness, thus consisting almost exclusively of epidermis, is used as a graft.

Harvesting of grafts:

The split–thickness graft is harvested after local anaesthesia, with razor blade/manual or motorized dermatome.

Recipient site preparation and grafting:

The recipient area is denuded by dermabrasion or by CO_2 laser. Grafts of approximately 4-5 cm length are placed over the denuded recipient site close to another with slight overlap. Grafts are held in place with staples or steri-strips, and covered with a non-adherent sterile dressing for approximately 1 week.

This technique gives good results provided the graft is very thin.

Advantages:

Suitable to cover large areas at one time

Suitable for difficult areas- eyelids and lips

Disadvantages:

Good skill is needed to take a uniformly thin graft

Not possible to cover palms, soles and body folds.

MATERIALS AND METHODS

Study Design

- Type of study: Non randomized prospective comparative study

Study Population:

- Sample Size: 50 patients divided into 2 groups

Study period:

July 2010- October 2011 (15 months)
First 6 months: Interventional period
Next 9 months: Follow-up period

Study analysis:
Results analysis- chi square test using spsf software

Place of study:

Department of Dermatology,
Government Stanley Medical College & Hospital,
Chennai.

From almost 400 vitiligo patients attending our OP (both old and new patients), 50 patients were selected according to the inclusion and exclusion criteria. They were enquired about the duration of their problem, any new lesions in the recent past or any new lesions occuring at the sites of trauma. They were examined meticulously and carefully looked for any erythema or evidence of koebnerization. Out of the 50

patients, 20 patients with mucosal vitiligo over lips, 28 patients with focal vitiligo over dorsum of foot/hand, eyelid, cheeks, forearm and legs, 1 segmental vitiligo and 1 acrofacial vitiligo patients were included in the study. They were given pre-treatment consultation, information sheet about the surgical procedure and fully explained about the pros and cons of the surgery. After their full understanding and willingness only they were enrolled in the study. Pre-operative assessment and investigations were done.

Pre-treatment consultation:

Patients were well informed about the method of the procedure, course of the treatment and possible adverse effects.

Informed and written consent were obtained.

A complete history regarding the onset, duration, any other co-existing systemic illnesses, and past history of any treatment taken for this condition were noted (ANNEXURE-I).

Proper counseling of the patient is very important. It should be emphasized that skin grafting cannot stop progression of the disease but only provides a pigmentary cover. The disease may recur. Immediate

results may not be cosmetically acceptable and color matching with surrounding normal skin can take up to 1-6 months.

PRE-SURGERY ASSESSMENT:

A thorough clinical examination as given in ANNEXURE-I was done.

Patients BCG scar was noted to look for Keloidal tendency.

Pre operative Work-up

- CBC, BT,CT, Platelet Count, Blood sugar
- Thyroid profile
- Screening for Hepatitis-B, VDRL, HIV testing
- Urine routine
- Serum Creatinine, Blood Urea Nitrogen, SGOT, SGPT
- Informed consent and photographs

INCLUSION CRITERIA

- Stable vitiligo
- Willingness to be enrolled in the study

EXCLUSION CRITERIA

- Active vitiligo
- Infection
- H/s/o Herpes labialis
- Keloidal tendency
- Bleeding disorders
- Overlying altered thick skin- due to PUVA
- Hypothyroidism
- Pregnant and lactating females

STUDY PROCEDURE:
MINIATURE PUNCH GRAFTING:

Instruments required:

- ❖ 2 and 2.5mm punches,
- ❖ Small Jeweler`s or graft holding forceps
- ❖ Small scissors

PROCEDURE PROPER[33-36]:

1) The donor and recipient area surgically prepared
2) Tray is kept ready
3) Informed consent obtained
4) Pre procedure photographs taken
5) 2% Lignocaine with or without adrenaline is infiltrated as local anaesthetic
6) The initial recipient chambers are made on or very close to the border of the lesion in order to minimize the achromic fissure.
7) The donor area is either upper lateral portion of thigh or gluteal region. In case of face, it is usually taken from the postauricular area.
8) In the donor area 2.5mm punch and in the recipient area 2.0 mm punch were used. This is done to prevent shrinking of the graft that is seen with same sized grafts.
9) The recipient area holes should be 1mm deeper than the thickness of the grafts to avoid cobblestone appearance.
10) The excess fat in the graft should be trimmed.

11) The grafts are placed directly from donor to recipient area. This speeds up the procedure and lessens the chance of infection. Care is taken so that the graft edges are not folded, the tissue is not crushed or placed upside down. The needle of the syringe or the tip of the scissors is used for proper placement of grafts in the recipient chambers.

12) Hemostasis is achieved by pressing a saline soaked gauze over the area.

11) For the recipient area three layers of dressing from inside out are:

Chlorhexidine tulle, sterile gauzepad and elastoplast bandage.

For the donor area also the same were applied.

12) One week of prophylactic systemic antibiotics given

13) Proper instructions for special areas like lips are necessary. To secure grafts recipient area these patients are advised to take liquid diet for the first 24 hours preferably with a straw. Patients are allowed to take normal diet after this period.

14) The dressings are opened after 24 hours to look for any dislodgement of grafts: if any are found, they are replaced.

15) Finally after 7 days the dressings are removed.

Post-op advice and Follow up:

- Limit the movements for the first 48 hours.
- After removal of the bandage, careful cleaning of the recipient area with gentle flushing of saline is advised.
- Avoidance of using soap or rubbing the area with towel for 2 weeks was advised. The area should be allowed to dry by itself.
- Post surgically the patients are exposed to sunlight for few minutes 2-3 times a week for about 3 weeks starting about 4th day after removal of bandage.
- Topical Mometasone, alternate days topical Mometasone 0.1% & Tacrolimus 0.1%, PUVASOL[37]/ PUVA/ NB-UVB[38] were tried in response poor patients.
- Patients were followed up fortnightly for first 2 months and then monthly.
- Patients were advised to report immediately if they have severe itching, pain, persistent swelling or erythema and purulent discharge from the lesion.

SPLIT-THICKNESS SKIN GRAFTING:

Instruments required:

Artery forceps

Sterile razor blade[39]

Small scissors

Jeweler`s forceps

Small bowl

Normal saline

Gauze pieces

Wooden board

Glass slides

Dermabrader

Diamond fraise

PROCEDURE PROPER[19]:

Harvesting the graft:

1) The donor area is prepared for the surgery

2) The required area is marked with sterile marking pen.

3) At least 10-25% larger area than the size of the vitiligo patch is marked, as there will be some contraction of the graft due to attached dermal elastic fibres.

4) Topical anaesthesia using a mixture of lidocaine 2.5% w/w and prilocaine 2.5% w/w cream under occlusion for at least 2 hours is kept.

5) After the cream is wiped off, a pin-prick test is performed to judge the degree of anesthesia. If the effect is not satisfactory, field block is given with 2% lignocaine at the margins.

6) The sterile razor blade is held with the help of artery forceps.

7) The donor site is firmly stretched with the help of left hand. The blade-holding artery forceps is moved to and fro with the help of the right hand and a thin split-skin graft is harvested. Closely packed fine bleeding points is seen after taking the graft.

8) The donor area is covered with a non-adherent dressing and a pressure dressing is given.

9) The recipient area is infiltrated with 2% lignocaine without adrenaline.

10) After surgical cleansing, the area is first marked with a surgical pen.

11) The skin is abraded with the help of dermabrader (either manual or electrical) a little deeper and it is stopped on seeing some coarse bleeding points.

12) The graft placed in the normal saline bowl is taken out and is stretched over the wooden board with the help of needle or Jeweler`s forceps. A glass slide is taken and is kept over the epidermal surface of the graft, so that the dermal side of the graft is free. Then the glass slide is kept over the recipient area and the dermal side of the graft is well placed over the denuded area. The free edge of the graft is stretched evenly at the periphery.

13) This is followed by a non-adherent dressing- chlorhexidine tulle, gauze piece and elastoplast.

14) One week of prophylactic systemic antibiotics given.

15) The dressing is removed at 24 hours to observe for any serous collection or hematoma, which is drained. Subsequently dressing is changed after a week.

16) The patient is then followed every fortnight for the first 2 months and monthly thereafter.

OBSERVATION AND RESULTS

Total number of Patients in the study group:

Study Population:

This study included 50 patients, 25 males and 25 females. The youngest in the study was 12 years of age and the oldest patient was 56 years of age. All satisfied the inclusion and exclusion criteria.

Table 1- Age wise distribution of the study population:

Age group	No. of patients
11-20 years	10
21-30 years	26
31-40 years	12
41-50 years	1
51-60 years	1

Chart no.1- age wise distribution of the study population

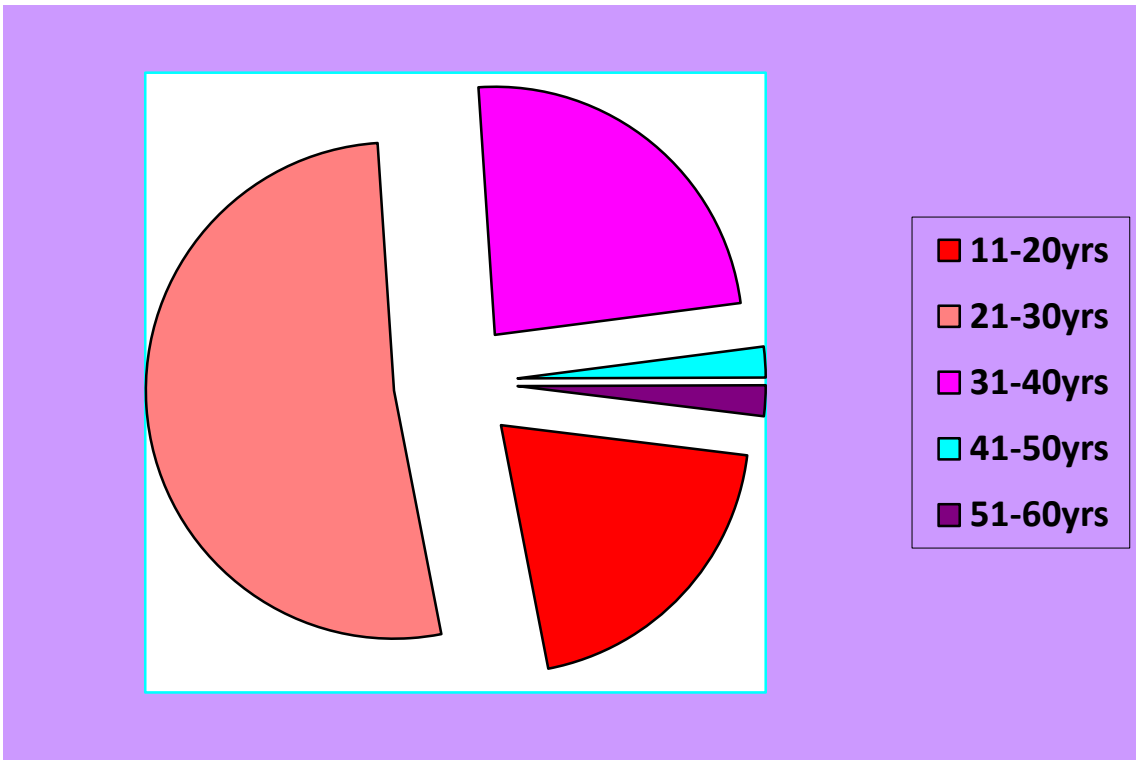
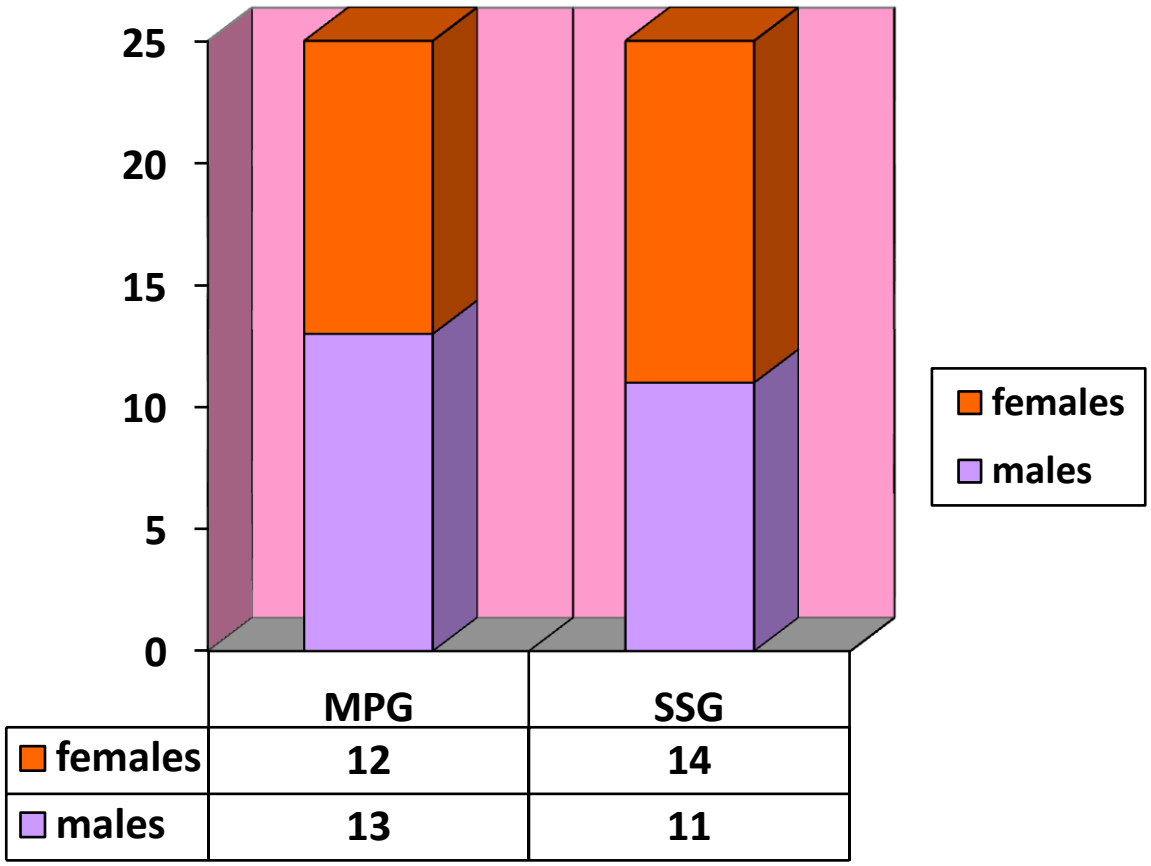


Table 2: Sex-wise distribution of the study group:

Name of the procedure	Males	Females	Total
Miniature punch grafting	13	12	25
Split skin grafting	11	14	25

Chart no. 2-sex-wise distribution of the study population

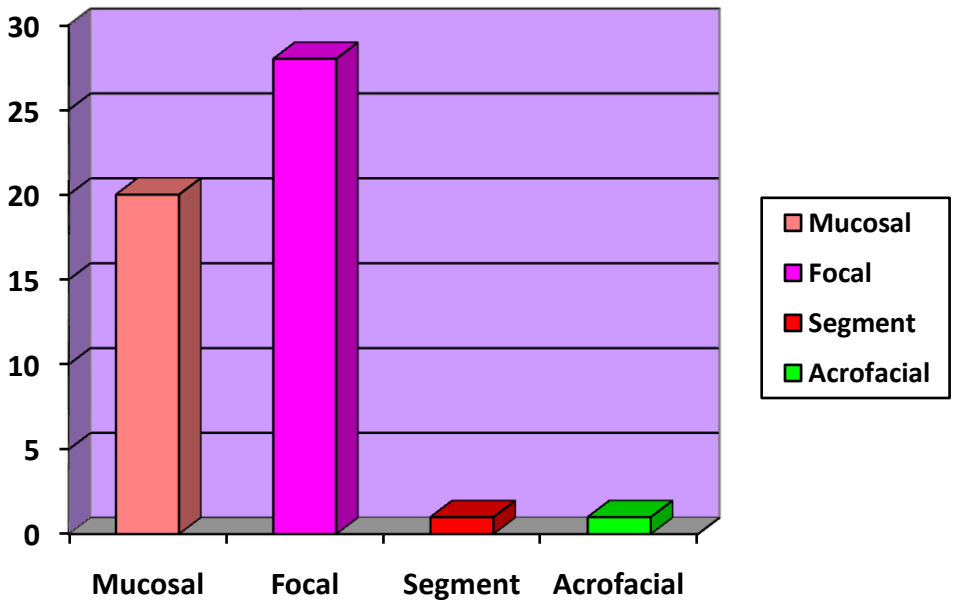


Out of the total 50 patients, 20 patients had mucosal vitiligo over lips, 28 patients had focal vitiligo over dorsum of foot/hand, eyelid, cheeks, forearm and legs, 1 patient had segmental vitiligo and 1patient had acrofacial vitiligo.

Table 3 – Vitiligo type included in the study:

S.No	Type of vitiligo	No.of patients
1.	Mucosal type	20
2.	Focal type	28
3.	Segmental	1
4.	Acrofacial	1
	Total	50

Chart No.3 – Vitiligo type included in the study



Various studies have chosen 6 months to 2 years as a period of stability. In this study, except 4 patients all others had vitiligo lesions ≥ 1year stable.

Miniature Punch grafting (MPG):

Out of the 25 patients, in about 18 patients the colour of the grafts changed gradually from brown, dark brown to black. The upper scab fell off by 10-15 days which left a pinkish colour. This pink colour changed to skin colour by almost 3 weeks. In the remaining 7 patients the graft appeared to be skin coloured through out. At about 4^{th} day after removal of the dressing all patients were asked to expose the grafted area for 3-5 minutes in the sunlight 2-3 times a week. After 1-1 ½ months uniform perigraft pigmentation started

appearing in 12 patients. So they were not given any other adjuvant therapy. In about 13 patients, the pigment islands remained static. For them adjuvant therapies like topical 0.1% Mometasone furoate were given for non-mucosal site and alternate days topical mometasone and topical 0.03% Tacrolimus were given to 4 patients with mucosal vitiligo. With the adjuvant therapy the lip vitiligo patients responded well. In the remaining 9 patients with non-mucosal vitiligo, 5 responded well with topical 0.1% Mometasone furoate. Out of the remaining 4, 2 were given

PUVA, 1 was given PUVASOL and 1 was given narrow band UV-B therapy. They all showed some response with adjuvant therapy. But one patient didn't respond to any adjuvant therapy and in fact he gradually showed koebnerization at the donor site and depigmentation at the recipient site. This patient actually had stable disease over the dorsum of foot for the past 2 years.

In the donor site, scarring was seen in 2 patients. Both were given topical steroids and showed some improvement. In the recipient site, immediate complication like graft displacement or rejection seen in 9 patients in 1 or 2 miniature grafts within 24 hours or at 7 days. Despite this melanocyte transfer was not affected and good amount of repigmentation seen in course of time without regrafting. 3 patients had hyperpigmentation and 1 had a variegated appearance. In subsequent follow up, they also improved. None of the patients had secondary infection.

Table4- Pigmentation response with miniature punch grafting:

% of improvement	Grading	No. of patients
< 25% response	Poor	1
25-50% response	Fair	1
50-75% response	Good	19
>75% response	Excellent	4

Split skin grafting (SSG):

The results of the procedure are noted in the form of perigraft pigmentary spread, intensity of the pigmentation and the colour matching with surrounding normal skin. Donor site scarring occurred in one patient. Hyperpigmentation in the recipient site was seen with 3 patients. Static graft with stuck on appearance occurred in one patient. Graft rejection or necroses at the edges were seen in 6 patients and in 2 of them the entire graft was shed down at about 14 days. Despite that 4 of them had excellent repigmentation and 2 of them had good amount of repigmentation.

Table 5- Pigmentation response with split skin grafting:

% of improvement	Grading	No. of patients
< 25% response	Poor	0
25-50% response	Fair	1
50-75% response	Good	13
>75% response	Excellent	11

In one patient with acrofacial type, the graft was static and hyperpigmented. It had a stuck on appearance and there were no pigment spread even after 9 months of follow up. One patient developed Keloidal scar at the recipient site and minimal scarring at the donor site. Three patients had hyperpigmentation at the recipient site. Six patients had milia which were removed by needling. Graft rejection and necrosis occurred in 6 patients. Out of them, 2 had almost complete necrosis and 4 had necroses at the edges. Despite that the pigmentation was good to excellent in them. None of the patients had either donor site or recipient site secondary infection.

Comparison between the results of MPG and SSG:

Response	MPG	SSG
Poor & Fair	2(8%)	1(4%)
Good & Excellent	23(92%)	24(96%)
Total	25	25

Crosstabs

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Procedure*Response	50	100%	0	.0%	50	100%

Procedure * Response Crosstabulation

			Response		Total
			≥Good	**Not good**	
Procedure	MPG	Count	**23**	**2**	**25**
		%within the procedure	**92.0%**	**8.0%**	**100.0%**
	SSG	**Count**	**24**	**1**	**25**
		% within the procedure	**96%**	**4.0%**	**100.0%**
Total		**Count**	**47**	**3**	**50**
		% within the procedure	**94%**	**6%**	**100.0%**

Chi-Square tests

	Value	Df	Asym.Sig (2 sided)	Exact.Sig (2 sided)	Exact.Sig (1 sided)
Pearson chi-Square	0.355[b]	1	0.552		
Continuity correction[a]	0.000	1	1.000		
Likelihood Ratio	0.361	1	0.548		
Fisher`s Exact Test				1.000	0.500
Linear-by-Linear Association	0.348	1	0.556		
N of Valid cases	50				

a. Computed only for a 2X2 table
b. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.50

The p value in this test is 0.552. Hence the difference between the results

of miniature punch graft and split skin graft is not significant in this study.

Incidence of complications in both the procedure:

Name of the complication	Miniature punch grafting	Split skin grafting
DONOR SITE:		
Infection	0	0
Scar	2(8%)	1(4%)
Koebnerization	1(4%)	0
Hyperpigmentation	0	0
RECIPIENT SITE:		
Infection	0	0
Hyperpigmentation	3(12%)	3(12%)
Cobbling	2(8%)	0
Variegation	1(4%)	0
Scar	2(8%)	1(4%)
Static graft	13(52%)	1(4%)
Depigmentation	1(4%)	0
Milia	0	6(24%)
Graft displacement/ rejection	9(36%)	6(24%)

Procedure * Scar Crosstabulation

			Scar	Total
			Present	Not	
Procedure	MPG	Count	2	23	25
		%within the procedure	8.0%	92.0%	100.0%
	SSG	Count	1	24	25
		% within the procedure	4.0%	96.0%	100.0%
Total		Count	3	47	50
		% within the procedure	6.0%	94.0%	100.0%

Chi-Square tests

	Value	**Df**	**Asym.Sig (2 sided)**	**Exact.Sig (2 sided)**	**Exact.Sig (1 sided)**
Pearson chi-Square	0.355[b]	1	0.552		
Continuity correction[a]	0.000	1	1.000		
Likelihood Ratio	0.361	1	0.548		
Fisher`s Exact Test				1.000	0.500
Linear-by-Linear Association	0.348	1	0.556		
N of Valid cases	50				

a. Computed only for a 2X2 table
b. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.50

The p value for this complication (scar) is 0.552 which is not <0.005. Hence

this is not significant.

Procedure * Static graft Crosstabulation

			Static graft	Total
			Present	Not	
Procedure	MPG	Count	13	12	25
		%within the procedure	52.0%	48.0%	100.0%
	SSG	Count	1	24	25
		% within the procedure	4.0%	96.0%	100.0%
Total		Count	14	36	50
		% within the procedure	28.0%	72.0%	100.0%

Chi-Square tests

	Value	**Df**	**Asym.Sig (2 sided)**	**Exact.Sig (2 sided)**	**Exact.Sig (1 sided)**
Pearson chi-Square	14.286[b]	1	.000		
Continuity correction[a]	12.004	1	.001		
Likelihood Ratio	16.281	1	.000		
Fisher`s Exact Test				.000	.000
Linear-by-Linear Association	14.000	1	.000		
N of Valid cases	50				

a. Computed only for a 2X2 table
b. 2 cells (.0%) have expected count less than 5. The minimum expected count is 7.00

The p value for this complication (static graft) is 0.000 which is <0.005. Hence it is significant. But the repigmentation improved with adjuvant therapy and the end result was not affected.

Procedure * Graft rejection Crosstabulation

			Graft rejection		Total
			Present	Not	
Procedure	MPG	Count	9	16	25
		%within the procedure	36.0%	64.0%	100.0%
	SSG	Count	6	19	25
		% within the procedure	24.0%	76.0%	100.0%
Total		Count	15	35	50
		% within the procedure	30.0%	70.0%	100.0%

Chi-Square tests

	Value	**Df**	**Asym.Sig (2 sided)**	**Exact.Sig (2 sided)**	**Exact.Sig (1 sided)**
Pearson chi-Square	.857[b]	1	.355		
Continuity correction[a]	.381	1	.537		
Likelihood Ratio	.862	1	.353		
Fisher`s Exact Test				.538	.269
Linear-by-Linear Association	.840	1	.359		
N of Valid cases	50				

a. Computed only for a 2X2 table
b. 2 cells (.0%) have expected count less than 5. The minimum expected count is 7.50

The p value in this complication (graft rejection) is 0.355 which is not <0.005. Hence it is not significant.

Focal vitiligo, dorsum of hand-Miniature punch grafting

focal vitiligo over dorsum of hand

after miniature punch grafting

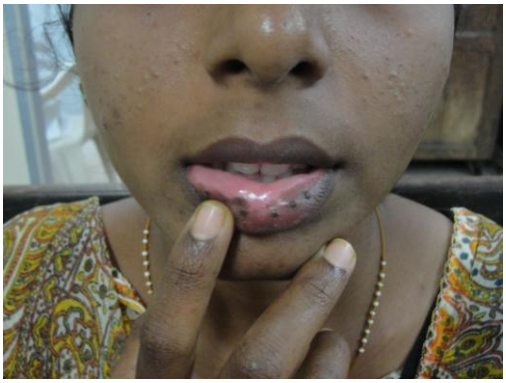
After 6 weeks

MINIATURE PUNCH GRAFTING LIP VITILIGO

2 weeks

6 weeks

8 weeks



10 weeks

SPLIT SKIN GRAFTING LIP VITILIGO

Immediate post op

After 3 weeks

SPLIT SKIN GRAFTING LIP VITILIGO

Before SSG

After SSG

After 4 weeks

FOCAL VITILIGO – DORSUM OF THE FOOT

Immediate post op

2 weeks

4 weeks

MINIATURE PUNCH GRAFTING LIP VITILIGO

Before MPG

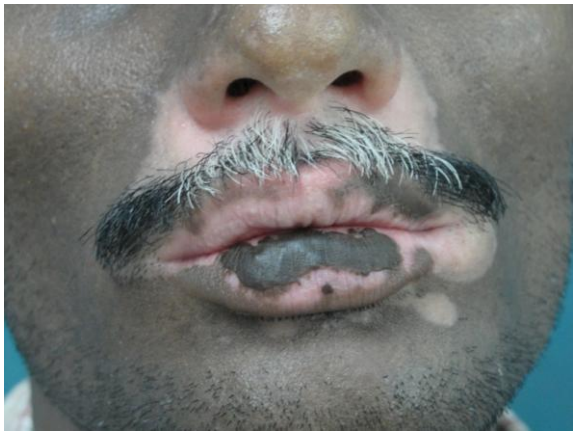
After MPG

After 4 weeks

After 8 weeks

SPLIT SKIN GRAFTING LIP VITILIGO

Before SSG



After 1 ½ months

COMPLICATIONS

Scarring at the donor site after MPG

Graft displacement After SSG

DISCUSSION

In spite of the considerable advancement in basic understanding of the disease process and the treatment protocol, Vitiligo has continued to elude researchers over the years. Conventional medical therapy remains beleaguered by unpredictable and inadequate outcomes. When the disease becomes refractory to conservative therapy, transplantation techniques are the only options left to replenish the lost melanocytes.

Among the various surgical methods for vitiligo, miniature punch grafting and split skin grafting are some of the simplest methods that can be easily done at a resource poor settings with a good result.

Miniature punch grafting:

In a study by S S Savant[36], he showed that 86% of cases had total repigmentation with excellent cosmetic colour match.

KG Singh[40] et al showed fair to excellent results in 87.5%.

In our study, 92% had good to excellent repigmentation. Vitiligo of any site and size can be treated by this method, with multiple sittings in bigger areas. In our study we chose mostly localized type with good stability of

the disease. Except 4 patients, all other had stability equal to or more than one year.

Cobblestoning, the most common side effect seen with punch grafting was initially seen in almost all cases up to the first month of follow up. But after 3 months it spontaneously disappeared in most of them and at last after 6 months it was seen only in 8% of cases. All the patients were advised sunexposure for 3-5 minutes twice a week. During assessment at 6 weeks, if the perigraft pigmentation if started well, they were not given any adjuvant therapy. If pigment spread is poor adjuvant therapy was given.

Falabella [5] had used 1-1.2mm punches in his study. In our study we had used 2-2.5mm punches with equally good results.

In the year 1972, Orentriech and Selmanwitz[43] showed maximum pigment spread as 1mm. Whereas Savant[8] in 1992, showed 15mm and Lahiri[34] et al in 2005 showed 12mm maximum pigment spread.

In our study we had an average of 10 mm maximum pigment spread without adjuvant therapy. With adjuvant therapy the average

maximum pigment spread was 14mm which was consistent with other studies.

Split Skin Grafting:

Split thickness skin grafting is very popular for its better colour, texture match, faster results and highest success mean rate.

Farah et al[41] reported satisfactory response with good colour match and minimal complications in 50 patients treated with split skin grafting.

In our study, among 25 patients 96% of them had good to excellent results. The pigment spread was noticed later as compared to miniature punch grafting, but the repigmentation was very good without any adjuvant therapy.

Somesh gupta et al[19] showed that it is best to dermabrade the recipient areas before the donor area is harvested. This gives some valuable time for the bleeding to stop at the recipient area and provides absolutely fresh graft.

Except for a bigger graft, for all other cases we also followed the same technique.

In our study except 3 patients, all others responded very well without any adjuvant therapy. With adjuvant therapy 2 responded well but the remaining one had hyperpigmentation at the graft site with no pigment spread at all.

CONCLUSION

- To conclude Miniature Punch grafting and Split skin grafting are easy to perform, cost effective procedure that gives excellent results with minimal side effect provided we choose the right candidate for treatment.
- Both these surgical methods do not require any laboratory technicians, expensive equipments or chemicals.
- The results obtained depended upon patient selection with achievement of excellent pigmentation when the lesions are stable, focal or segmental and over areas with minimum mobility.
- Miniature punch grafting is not only an easy, safe and least expensive method, but it is one of the most effective treatment options in treating stable and recalcitrant vitiligo.
- Cobblestoning, the commonest side effect with punch grafting, can be minimized by using miniature punch grafting technique using 2-2.5mm punches.
- Though miniature punch graft is easy and safe, pigment dispersion takes a longer time to achieve. So immediate cosmetic results are less compared to split skin grafting hence patient preference is more for the latter.

- This problem can be minimized by the use of adjuvant therapies.
- Split thickness skin grafting gives almost immediate results, but requires expertise in harvesting the graft.
- Pigmentation is uniform with split thickness skin grafting.
- Cobblestoning, common with minigrafting, does not occur in SSG.
- Adjuvant therapy is not routinely needed for split skin grafting
- Graft necrosis though is more common with split skin grafting, but repigmentation was also observed in such grafts due to the adjuvant effect of dermabrasion done for preparation of the graft site.
- The most significant factor influencing success with split skin graft was mobility of the recipient site with poor results at highly mobile recipient sites.
- Thus, even in the absence of adequate resources, both the surgeries can be done with almost similar good results.

	Miniature Punch grafting	Split Skin grafting
Success rate	92%	96%
Cobbling	+ (minimized by using smaller punches)	-
Milia	-	+
Graft rejection	+	++++
Immediate results	No	Yes
Uniform pigment dispersion	No	Yes
Cosmetically	Good	Excellent
Palms & soles	Useful	Not useful
Mobile areas	Can be done	Very difficult to maintain the graft
Adjuvant therapy	Needed in many	Usually not needed

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ANNEXURES

PROFORMA

- NAME: AGE: SEX:

- OCCUPATION: INCOME:
- CHIEF COMPLAINTS:

- History of present illness:

- Past history:
- Family history:
- Personal history:
- Examination:

DIAGNOSIS:

CONSENT FORM

- Mr./Mrs./Miss
- Age
- Address
- Phone:
- Name of the procedure
- I undersigned Mr./Mrs./Miss
-

Have been explained regarding above said procedure in my regional language. I am fully aware of the possible side effects and risks involved in this procedure. I am also aware that this particular procedure may not always be successful and no guarantee can be made for successful outcome of such procedure.

I have explained that this procedure will be performed by Dr.T.Vanathi.

I also given my consent that during this procedure if any complication arises, I may be given any emergency treatment best suitable to me without asking my prior permission.

I further state that I have carefully read and understood all the information provided in this form and under fully conscious mind I hereby give my written consent for the said procedure with its risks involved.

Signature of Patient/thumb impression
Signature of Parents/Guardian(for minors)
Name &Relationship if signed by other than parent

Witness:

Name:
Signature:

Date: